Aquaculture 459 (2016) 137–142

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effects of reduced pH on the early larval development of hatchery-reared Donkey's ear abalone, *Haliotis asinina* (Linnaeus 1758)

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ARTICLE INFO

Article history:
Received 20 September 2015
Received in revised form 29 February 2016
Accepted 13 March 2016
Available online 15 March 2016

Keywords:
Tropics
Ocean acidification
Larval survival
Abalone
Calcification
Trochophore

ABSTRACT

Declining pH levels caused by absorption of accumulating atmospheric CO_2 in the ocean threaten the normal development of early life stages and particularly impair the ability of calcifying organisms to construct their shells. An experiment was conducted to determine the direct effects of reduced pH on % hatching of fertilized eggs, survival and developmental stages of trochophore larvae of the Donkey's ear abalone, *Haliotis asinina*. The ambient pH (7.97 ± 0.02) of experimental seawater was reduced by bubbling food-grade CO_2 to obtain the desired pH levels of 7.78 ± 0.03, 7.60 ± 0.03 and 7.40 ± 0.02 as treatments. There were increasing negative impacts of reduced pH on the mean % hatched trochophores (97.6 at ambient pH conditions, 83.9 at pH 7.78, 24.1 at pH 7.60, and 1.4 at pH 7.40). Significant impacts of reduced pH were also observed on mean % survival of trochophores (98.3 at ambient pH conditions, 84.9 at pH 7.78, 24.1 at pH 7.60, and 1.4 at pH 7.40). Of the surviving trochophores, 23.2 ± 3.2% (mean ± sd) of those exposed to pH 7.60 were morphologically deformed but in much lower pH treatment (pH 7.40), all the trochophores were deformed. At pH 7.78, only 63.3 ± 3.7% of the surviving trochophores developed normally compared to 96.7 ± 1.6% normal trochophores at ambient pH conditions. Normal trochophores have prototrochal cells and girdle fully developed and developing shell tissue. Malformed trochophores showed highly undefined morphological characters and cleavage failed to progress to further developmental stages. In conclusion, the early larval development of *H. asinina* was found to be highly sensitive to reduced pH levels projected for the end of the present century. Therefore, future rise in CO_2 concentration in tropical marine waters will likely pose a significant threat to the natural population densities of this species.

Statement of relevance:

- To understand how lower pH levels can affect this high-value haliotid species is critical because of the importance of this species to fishery and mariculture production;
- The deleterious effects of reduced pH on the early larval development of (up to hatch-out stage) of the tropical abalone (*H. asinina*) were demonstrated in this study;
- A significant decrease in hatching rates, increased larval mortality, delayed and abnormal development will most likely have great impact on the population dynamics of this species in their natural habitat;
- All the observations in this study suggest that reduced pH can affect early larval development and survival of abalone in the coastal ecosystem; and
- these negative effects on larvae may persist into juvenile and adult stages of abalone which would likely contribute to a severe reduction of their natural populations.

1. Introduction

Recent studies (i.e., Harrould-Kolieb et al., 2010; Ishimatsu and Dissanayake, 2010; UNEP, 2010; Byrne, 2011) show that the accumulation of CO_2 in the Earth's atmosphere has lead to an increase in partial pressure of carbon dioxide (pCO_2) in the oceans and a corresponding decrease in pH, which is now popularly called "ocean acidification". Lowering pH levels increase ocean acidity and impair the ability of shell-forming organisms (i.e. shellfish, plankton, and corals) to construct their shells (Kleypas et al., 2006; Crim et al., 2011; Wright, 2011; Andersen et al., 2013). A comprehensive review of current literature on the effects of ocean acidification on all life stages (early life history to adult) of shelled molluscs revealed that embryonic and larval

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http://dx.doi.org/10.1016/j.aquaculture.2016.03.027
0044-8486/

development of shelled molluscs are highly sensitive to elevated pCO_2 (reduced pH) as evidenced by size reduction, decreased larval survival, increased number of deformed larvae and delayed larval development (Gazeau et al., 2013). Hence, ocean acidification is a major threat to the larvae of calcifying organisms as it decreases the availability of carbonate ions required for skeletogenesis and it also exerts a direct pH effect on the physiology of the organisms (Byrne, 2011).

The predicted decrease in pH of the ocean's surface waters by 0.3–0.5 units by the year 2100 (Caldeira and Wickett, 2003) would likely affect the sensitive and vulnerable early developmental stages of abalones and other calcifying molluscs because these life stages have specific environmental requirements. There is, however, limited information regarding the effects of reduced pH on tropical molluscs particularly on early life stages of abalone species. Where studies exist, they are concerned mainly with bivalves in the temperate region (i.e. Talmage and Gobler, 2011; Barton et al., 2012; Dickinson et al., 2012; Fernandez-Reiriz et al., 2012; Timmins-Schiffman et al., 2012; Andersen et al., 2013). Only recently a few studies on the effects of reduced pH on larval settlement, growth and survival of *Haliotis asinina* larvae were reported from the tropical area (Tahil and Dy 2015a and 2015b). Abalone cultured in low pH conditions (pH 7.6) showed shell erosion which is characterized by a shiny appearance due to dissolution of the upper prismatic layer, revealing the inner nacreous layer (Wright, 2011). Exposure to reduced pH (7.16) also caused alterations in kidney and gill definition, tubule and lumen size, and an increase in gill hyperplasia and abnormalities in juvenile greenlip abalone, *Haliotis laevigata* and blacklip abalone, *Haliotis rubra* (Harris et al., 1999).

These negative effects of reduced pH may threaten the sustainability of aquaculture production of this species. Like any other abalone species, the free-swimming trochophores of *H. asinina* are likely more susceptible to expected changes in seawater chemistry, which may result in a significant reduction in larval production in the hatchery system. Therefore, there is a need to understand how lower pH levels can affect the early life stages of this species. As part of the studies conducted by Tahil and Dy (2015a and 2015b), the present experiment was conducted to determine the direct effects of reduced pH levels on the hatching rate, development and survival of early trochophores (before settlement) of the Donkey's ear abalone under hatchery conditions.

2. Materials and methods

2.1. Experimental design

The experiment was conducted at the Tawi-Tawi Multi-Species Hatchery in Latu-Latu, Bongao, Tawi-Tawi, Philippines in March and April 2013 using a randomized complete block design. An epoxy-coated wooden tank with eight compartments (4 × 2) and aeration system was used for the experiment. Each compartment has a dimension of 60 × 60 × 60 cm. The experiment was conducted in four successive trials with two replicates for each treatment per trial. These four trials simulated the successive spawning of abalone broodstock within three to five days before or during the new moon and full moon, with an interval of 13–26 days (Singhagraiwan and Doi, 1992; Capinpin, 1995; Capinpin et al., 1998). Part of the water quality management was the siphoning of water near or at the bottom of the tank to remove the discharged egg membranes and unhatched eggs, and each tank compartment was provided with fresh/new filtered and UV-treated seawater (without added CO_2) during each trial.

2.2. pH manipulation and maintenance

The ambient pH levels were first recorded for seawater pumped from the subtidal area of Tawi-Tawi Bay for use in the hatchery. The Bay is partly surrounded by mangrove forest and where pH levels are expected to be relatively low and variable compared to the open ocean. *H. asinina* is the most common in the shallow reef flat (ca. 0.5–4.5 m depth) around Tawi-Tawi Bay where they may be exposed to such variations in pH levels in nature. The mean pH of the ambient seawater was 7.97 ± 0.01 (Control). Based on the preliminary simulation to determine the amount of CO_2 needed to maintain the pH within the range, a food-grade CO_2 was added every 2 h for an average duration of 7.5–54 s depending on the desired pH level for each treatment. After adding CO_2, the ambient pH level of the seawater was reduced to pH 7.78 ± 0.02 (Treatment I), pH 7.60 ± 0.02 (Treatment II), and pH 7.40 ± 0.01 (Treatment III). The reduction of ambient pH to these levels were based on the expected decrease of 0.3–0.5 units in the ocean pH by 2100 (Caldeira and Wickett, 2003 and 2005). The pH and all other water parameters were also measured every 2 h before and after the CO_2 diffusion. The pH and temperature were measured using a pH meter (Orion 230Aplus, Orion Research, Inc., U.S.A.; Precision: 0.05 pH units) calibrated manually with two pH buffer solutions (4.01 and 7.00) using an ATC probe. Salinity and dissolved oxygen were measured with a refractometer (ATAGO 100-S, S/Mill-E, Japan) and DO meter (Orion M810Aplus, Orion Research, Inc., U.S.A.), respectively.

Total alkalinity (TA) of experimental seawater was measured using an alkalinity titration kit (Hanna Instruments, Woonsocket, USA). Concentrations of CO_2, carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-), partial pressure of CO_2 (pCO_2), saturation state of calcite (Ω_{calc}) and aragonite (Ω_{arag}) were then calculated from measured TA and pH using the software CO_2SYS (Pierrot et al., 2006) and by using the dissociation constants of carbonic acid from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) (Moulin et al., 2011).

Mean water temperatures in all treatments ranged from 26.0 ± 0.1–26.3 ± 0.4 °C, salinity from 34.6 ± 0.3–35.1 ± 0.2 psu while dissolved oxygen varied between 7.9 ± 0.2 and 8.2 ± 0.5 mg L^{-1} (Table 1). The carbonate chemistry of experimental seawater is also shown in Table 1. Carbonate ion concentration was lowest (59.3 μmol kg^{-1}) in the reduced pH treatment (7.40) relative to ambient conditions (221.4 μmol kg^{-1}). The ambient pCO_2 (453 μatm) was similar to the reports of other authors for molluscs from the Pacific area [e.g., 400 μatm (Crim et al., 2011); 468 μatm (Timmins-Schiffman et al., 2012); 469 μatm (Andersen et al., 2013)].

2.3. Collection and stocking of fertilized eggs

One hundred sixty eight broodstocks (47 males and 121 females) of *H. asinina* (6–9 cm shell length) collected in February 2013 from their natural habitat around Tandoh Island, Tawi-Tawi Bay, southern Philippines were stocked in concrete tanks and allowed to spawn spontaneously under hatchery conditions. A new group of fertilized eggs from the same group of broodstock was used during each trial. The response variables for this experiment were % hatching rate, % normal hatched trochophores, and % survival. Fertilized eggs from natural spawning in the hatchery were collected into an 80 μm sieve installed at the drain outlet of the spawning tank. Collected eggs were washed with filtered seawater to remove excess sperm and placed in 5-liter rectangular plastic containers. Then, egg densities were sampled by taking three 10-mL subsamples using a pipette and placed in a watch glass. The eggs were counted under a monocular 40× microscope (Cole Parmer, Vernon Hills, Illinois) using a Sedgewick Rafter Counting Chamber. Each tank compartment (with 100-liter filtered seawater) was stocked with 3000 fertilized eggs which were allowed to hatch and undergo morphogenesis up to late trochophore stage. Immediately after stocking of fertilized eggs within the tank, the pH was gradually adjusted through CO_2 diffusion (Section 2.2) to the desired level for a particular treatment.

2.4. Hatching and larval development

Exposure of fertilized eggs to these pH levels was done for only 6.4 h since hatching in *H. asinina* will take place from 5–6 h after fertilization (Singhagraiwan and Doi, 1993; Capinpin et al., 1998), and the